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Region VI of Cauliflower Mosaic Virus Encodes a Host Range Determinant

JAMES SCHOELZ, ROBERT J. SHEPHERD,* AND STEPHEN DAUBERT
Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

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A domain of cauliflower mosaic virus (CaMV) which controls systemic spread in two solanaceous hosts (*Datura stramonium* and *Nicotiana bigelovii*) was mapped to the first half of open reading frame 6. Whereas ordinary strains of CaMV are unable to infect solanaceous species except to replicate locally in inoculated leaves, a new CaMV strain (D4) induces chlorotic local lesions and systemically infects both *D. stramonium* and *N. bigelovii*. To determine which portion of the CaMV genome controls systemic spread of the virus in solanaceous hosts, nine recombinant genomes constructed between D4 and two ordinary strains of the virus were tested for their ability to infect solanaceous hosts. A 496-base-pair DNA segment comprising the first half of open reading frame 6 specified the type of local lesions and systemic spread of the virus in solanaceous hosts. Exchange of this segment of the genome between strains of CaMV converted a compatible host reaction to an incompatible (hypersensitive) one in response to infection. This suggests that the gene VI protein interacts with the plant to suppress hypersensitivity, the normal response of solanaceous hosts to CaMV infection.

The genome of cauliflower mosaic virus (CaMV) consists of circular, double-stranded DNA approximately 8,000 base pairs (bp) in length (26). This genome and its coding potential have proven amenable to detailed analysis on the molecular level. Several isolates of CaMV have been cloned in infectious form and characterized by restriction endonuclease mapping (18, 19). The genomes of three of these isolates have been completely sequenced, revealing a coding capacity organized into six major open reading frames (ORFs) and two intergenic regions (2, 11, 12). Protein products or functions have been assigned to three of the ORFs. Insertional mutagenesis, as well as exchange of DNA segments between cloned strains, has demonstrated that ORF 2 encodes a product required for insect transmission (1, 9, 33). The coat protein gene was mapped to ORF 4 by use of an expression vector in a bacterial system (7). Cell-free translation studies on mRNAs isolated from infected plants have shown that ORF 6 may code for an inclusion body protein (6, 22). In addition, ORF 5 likely encodes viral reverse transcriptase (24, 28, 30).

In an attempt to assign other functions to the CaMV genomic map, we have been studying the ability of several CaMV strains to infect solanaceous plants. The host range of CaMV was originally reported to be limited to crucifers (29, 31). Strains of CaMV have since been found to cause necrotic local lesions on *Datura stramonium*, a solanaceous host (20). Electron microscopy of tissue surrounding the necrotic lesions has shown that the virus can replicate and form inclusion bodies in *D. stramonium*, but systemic spread is blocked. Recently, a new strain of CaMV that develops systemically in several solanaceous hosts has been described (25a). This strain, designated D4, causes chlorotic rather than necrotic local lesions on *D. stramonium*, followed by systemic development of the virus throughout the plant.

In a preliminary investigation (8), recombinant virus genomes were constructed using cloned segments from different virus strains. In that case evidence was obtained that some sequence located between the middle of ORF 5 and the *Bst*EII site in the large intergenic region (see Fig. 1) controls systemic development in solanaceous plants. The specific region responsible for the increased host range was not identified. In this paper, we report the construction and host range behavior of several more recombinant viruses derived from D4 and two ordinary strains of CaMV. We found that the source of ORF 6 determines the type of local symptom, i.e., whether necrotic or chlorotic lesions are produced in response to infection. In addition, ORF 6 controls the capacity for systemic development of the virus in solanaceous hosts. In this case the genetic domain governing host reaction and systemic virus movement has been associated with a much smaller region of the genome, viz., a 496-bp segment of ORF 6 near the N terminus of the protein.

MATERIALS AND METHODS

Materials. Restriction enzymes and T4 DNA ligase were purchased from New England BioLabs, Inc. (Beverly, Mass.), and were used as directed by the supplier.

Bacteria and media. The bacterial strains used for cloning experiments was *Escherichia coli* 71-18 (21). *E. coli* was grown in 2YT liquid medium or on 2YT solid agar at 37°C and transformed by the procedure of Cohen et al. (5). Kanamycin and ampicillin were both used at a concentration of 100 μg/ml for selection of transformants and maintenance of pBR322 and pUC13 in *E. coli*. For transformation with pUCD9x (4), kanamycin and ampicillin concentrations were 30 and 20 μg/ml, respectively.

Purification of DNA. Plasmid DNA was purified from *E. coli* by the procedure of Holmes and Quigley (16). CaMV viral DNA was purified from infected plants by the method of Gardner and Shepherd (13). CaMV was purified from infected plants as described elsewhere (R. Richins and R. J. Shepherd, Phytopathology, in press).

Description of cloned viral strains. Three strains of CaMV were used for the construction of recombinant viruses. D4 is a strain of CaMV that can systemically infect several solanaceous hosts, including *D. stramonium* and *Nicotiana bigelovii*. The complete genome of CaMV D4 has been cloned in infectious form into the *Sal*I site of pBR322 (25a). CaMV strains CM1841 and Cabb-B are both unable to infect *D. stramonium* and *N. bigelovii* systemically under green-

* Corresponding author.

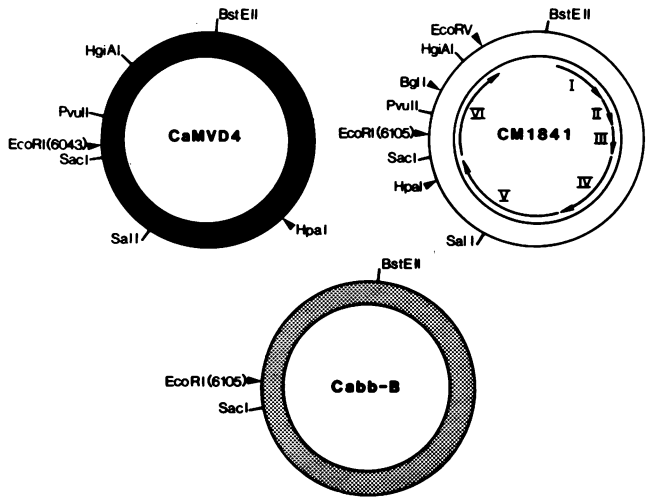

FIG. 1. Restriction enzyme maps of CaMV D4, CM1841, and Cabb-B. The positions of the six open coding regions are indicated on the restriction enzyme map of CM1841, which has been completely sequenced (12). The circular maps are oriented with the zero position at the top. Restriction enzyme sites which were used for exchanges of DNA segments are indicated by bars. Sites that were used for identifying a DNA segment are indicated by arrowheads.

house conditions (20, 31). Cabb-B has been cloned into the *Xho*I site of pGJ1, and the clone has been designated pCaMV12a (7). CM1841 has been cloned into the *Sal*I site of pBR322, and this clone has been designated pCaMV10 (12). Recombinant viral clones pCaMVH7, pCaMVH8, pCaMVH9, and pCaMVH10, cloned into the *Sal*I site of pBR322, have been previously described (8).

Host response analysis. The recombinant viruses and the three CaMV strains were initially inoculated to turnip seedlings (*Brassica campestris* var. Just Right). Four to six weeks after inoculation, virus was partially purified from infected turnips. The purification protocol was a modification of the procedure used for DNA isolation. Approximately 30 g of infected turnip leaves was homogenized in 180 ml of CaMV grinding buffer (200 mM Tris hydrochloride, pH 8.0, 20 mM EDTA, pH 8.0, 1.5 M urea). Triton X-100 was added to 2.0% from a 25% stock solution, and the mixture was stirred for 30 min at 4°C. The solution was then centrifuged at 12,000 × *g* for 10 min. The supernatant was filtered through two layers of Miracloth and then centrifuged at 150,000 × *g* for 1 h at 4°C. The pellet was suspended in 4 to 8 ml of TE (10 mM Tris hydrochloride, pH 8.0, 1 mM EDTA, pH 8.0). This concentrated virus sample was used as the inoculum for the solanaceous hosts *D. stramonium* and *N. bigelovii* at the three- to six-leaf stage. *D. stramonium* was then placed in a growth chamber which was set for an 8-h day at 18°C. *N. bigelovii* was tested in the greenhouse held at about 23°C under natural light. Symptoms were scored through 7 weeks in both cases.

Serological tests. Antiserum against Cabb-B virus was prepared previously (14). The enzyme-linked immunosorbent assay of Clark and Adams (3) was modified by substitution of a horseradish peroxidase-immunoglobulin G conjugate (32) for the alkaline phosphatase-immunoglobulin G conjugate. Gamma globulin was coated onto Immulon 1 plates (Dynatech Laboratories, Inc., Alexandria, Va.) at a concentration of 1.0 μg/ml. Leaf disks were collected randomly from various leaves of test plants with a 9-mm (diameter) cork borer and ground at a tissue-buffer ratio of 1:10 (wt/vol). Samples were then diluted three- to fourfold before being loaded onto the polystyrene plates. Horseradish peroxidase-immunoglobulin G conjugate was cross-reacted against healthy plant sap and then added to plates at a concentration of 1.0 μg/ml. The incubations of immunoglobulin G, tissue samples, and conjugate were at 37°C for 90 min. Plates were washed three times with phosphate-buffered saline-Tween between loadings. The substrate, *o*-phenylenediamine, was then added at a concentration of 0.7 μg/ml and allowed to react for 10 min before the absorbance was read. A_{450} was read on a Titertek Multiskan photometer (Flow Laboratories, Inc., McLean, Va.).

RESULTS

Construction of recombinant viruses. The restriction enzyme maps of D4, CM1841, and Cabb-B are illustrated in Fig. 1. The viruses have several restriction enzyme sites in common, which facilitated the exchange of DNA segments between them. Any particular DNA segment could be identified by the presence or absence of a characteristic restriction enzyme site within the segment.

The compositions of the recombinant viruses are illustrated in Fig. 2. Recombinant viruses were constructed in one of two ways. In the first strategy, DNA segments were exchanged between two cloned strains, and the recombinant viral clone was identified from the parent clones by its characteristic restriction enzyme map. The recombinant viral DNA was then excised from the vector and inoculated into turnip seedlings (*B. campestris* var, 'Just Right') to initiate infection. Most of the recombinant viruses were made in this manner.

In the second strategy, DNA segments were first subcloned into a bacterial vector, and pairs of subclones were selected which would constitute the recombinant viral genome. The vectors were excised, and the complementing DNA fragments were inoculated onto turnips to establish

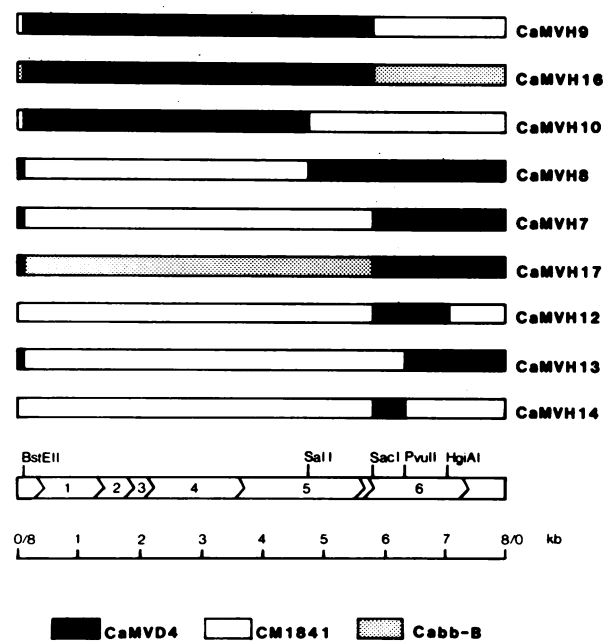

FIG. 2. Compositions of the nine recombinant viral genomes. The genomes are presented in a linear fashion to facilitate comparison between them. The zero position of the circular map (Fig. 1) corresponds to each end of these linear diagrams. kb, Kilobases.

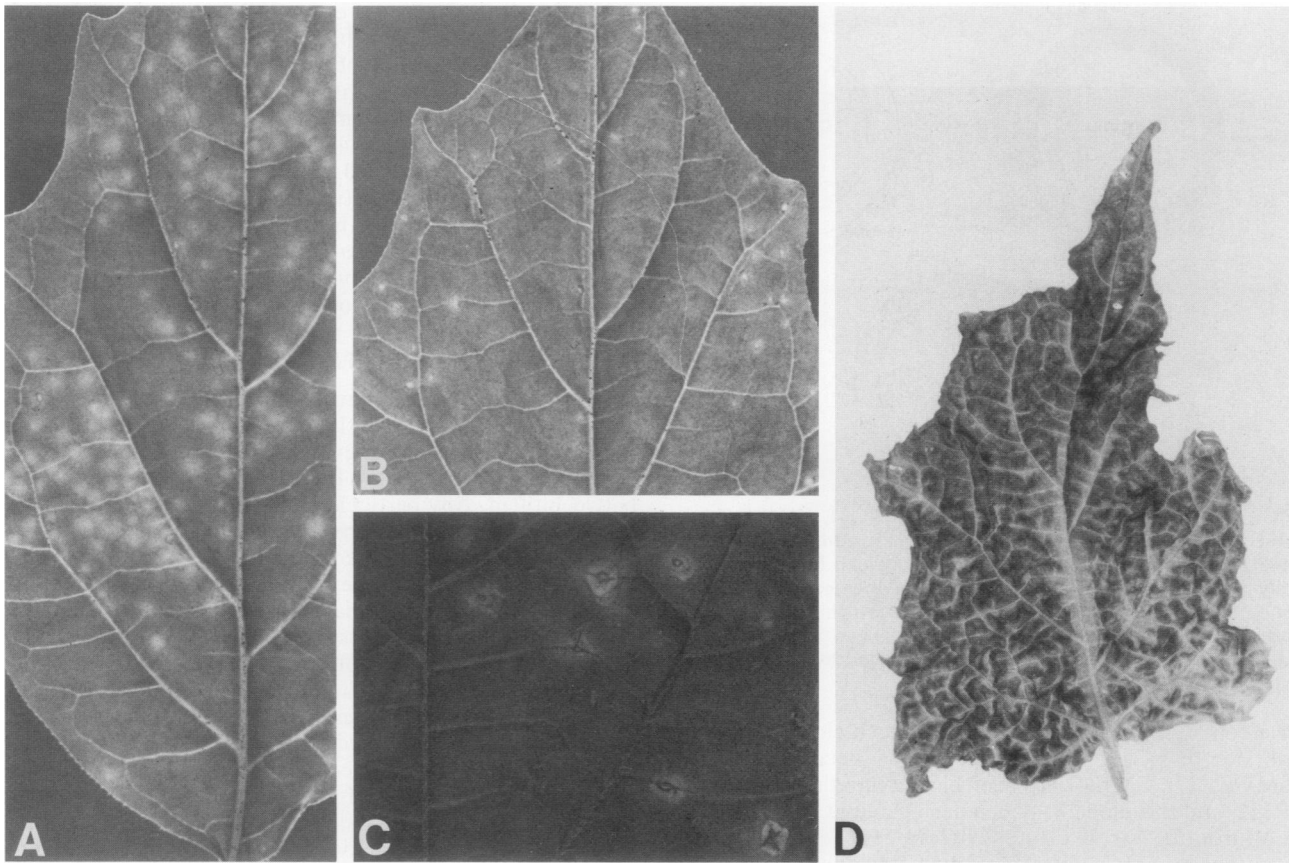

FIG. 3. Response of *D. stramonium* to infection by CaMV D4, CM1841, and Cabb-B. A, D4 chlorotic local lesions; B, Cabb-B necrotic local lesions (CM1841 local lesions are identical to those caused by Cabb-B); C, Cabb-B systemic symptoms; D, D4 systemic symptoms.

infection. CaMV H13, H14, and H17 were made in this way. In all cases, the presence of the recombinant virus in turnips was confirmed by isolating viral DNA from plants and mapping key restriction enzyme sites.

Construction of pCaMVH12. Full-length CM1841 DNA was isolated from infected turnips, linearized by cleavage with *Sac*I, and then ligated into the *Sac*I site of pUC13. The resultant clone was linearized by partial digestion with *Sal*I, and a DNA segment containing the kanamycin resistance gene derived from Tn*903* (9) was ligated into the unique viral *Sal*I site. The kanamycin-resistant construct and pCaMVD4 were cleaved with *Hgi*AI (*Hgi*AI cleaves pUC13 and pBR322 at many sites), and a pUC13 derivative which lacks a *Sal*I site (owing to removal of the *Sma*I-*Hinc*II DNA segment) was cleaved with *Sac*I. The three digests were mixed and ligated together, and *E. coli* was transformed. Recombinant viral clone pCaMVH12-kan was identified by screening Ap^r Km^r transformants for full-length viral clones bearing an *Eco*RI site at position 6040 and an *Eco*RV site at 7340 (CM1841 coordinates; 12). The *Sal*I segment bearing the gene for kanamycin resistance was then removed by *Sal*I cleavage.

Construction of CaMV H13 and H14. Recombinant viruses CaMV H13 and H14 were derived from pCaMV10 and recombinant viral clone pCaMVH7. A *Sal*I-*Pvu*II double digest of either pCaMV10 or pCaMVH7 DNA yields two viral DNA segments which are 6,546 and 1,485 bp in length. Both *Pvu*II-*Sal*I fragments were subcloned into pBR322. The smaller subclone derived from pCaMV10 was mixed with the larger subclone from pCaMVH7, and viral DNA segments were excised from the vectors by digestion with *Sal*I and *Pvu*II. The two viral DNA segments, which together constitute the genome of CaMV H13, were coinoculated onto young turnips at a concentration of 0.5 μg/μl. Ligation of the DNA fragments occurred in the plant to form an infectious recombinant virus. CaMV H14 was made in a similar manner by starting with the larger subclone derived from pCaMV10 and the smaller subclone from pCaMVH7. The identities of CaMV H13 and H14 were confirmed by isolating viral DNA from infected turnips and mapping characteristic restriction enzyme sites. Recombinant CaMV H13 viral DNA had an *Hpa*I site at position 5514 and an *Eco*RI site at 6105 and lacked a *Bgl*I site at 6656. Recombinant CaMV H14 had an *Hpa*I site at 5514, an *Eco*RI site at 6043, and a *Bgl*I site at 6656.

Construction of pCaMVH16. pCaMVH8 and pCaMV12a were cleaved with *Bst*EII and *Sac*I, mixed, and ligated. *E. coli* cells were transformed with the ligation mixture, and an intermediate construct, a pCaMVH8 derivative with its *Sac*I (5822) to *Bst*EII (126) section derived from pCaMV12a, was identified by the presence of an *Eco*RI site at position 6105 and an *Xho*I site at 1642. This hybrid, designated pCaMVH15, consisted of a CM1841 segment from *Bst*EII (126) clockwise to *Sal*I (4833), a D4 segment from *Sal*I (4833) to *Sac*I (5822), and a Cabb-B segment from *Sac*I (5822) to *Bst*EII (126). pCaMVH15 and pCaMVD4 were mixed, cleaved with *Bst*EII and *Pvu*I (*Pvu*I cleaves only within the pBR322 vector), and ligated. pCaMVH16 was identified

TABLE 1. Disease symptoms and virus concentration in *D. stramonium* after inoculation with recombinant strains of CaMV

CaMV strain	Test 1			Test 2			Test 3		
	Local reaction	Systemic reaction	A_{450}	Local reaction	Systemic reaction	A_{450}	Local reaction	Systemic reaction	A_{450}
D4	10C[a]	10[b]	0.492[c]	10C[a]	10[b]	1.024[c]	10C[a]	10[b]	0.310[c]
Cabb-B	10N	4[d]	0.032	10N	0	0.032	10N	10[d]	0.028
CM1841	10N	0	0.017	6N	0	0.016			
H9	10N	0	0.007	10N	0	0.028	10N	1[d]	0.036
H16	10N	0	0.009	10N	0	0.013	10N	1[d]	0.021
H10	10N	0	0.012	10N	0	0.003	10N	0	0.012
H8	10C	10	0.805	10C	10	2.326			
H7	10C	10	0.787	10C	10	2.262			
H17	10C	10	0.269	10C	10	0.167			
H12	10C	10	0.321	10C	10	2.281			
H13	10N	9[d]	0.006	10N	0	0.013	10N	0	0.025
H14	10C	10	0.421	10C	10	0.859			
None	0	0	0.007	0	0	0.057	0	0	0.015

[a] Number of plants of 10 inoculated that gave the indicated reaction 2 to 3 weeks after inoculation. C = Chlorotic local lesions; N = necrotic local lesions.
[b] Number of plants of 10 inoculated that reacted with systemic symptoms 4 to 5 weeks after inoculation.
[c] Virus concentration was determined from tissue samples taken 6 to 7 weeks after inoculation.
[d] Symptoms consisted of necrotic or chlorotic spots scattered on the older leaves (see the text).

among the resulting transformants by the presence of an *Eco*RI site at position 6105 and an *Hpa*I site at 3050 and by lack of an *Xho*I site at 1642.

Construction of CaMV H17. Digestion of CaMV H7 or Cabb-B uncloned viral DNA with *Bst*EII and *Sac*I yields two fragments, which were 5,697 and 2,335 bp in length. The 2,335-bp fragment of CaMV H7 and the 5,697-bp fragment of Cabb-B were isolated by subcloning into pUCD9x. The two clones were mixed, cleaved with *Bst*EII and *Sac*I to excise the vector, and inoculated onto turnips. Viral DNA recovered from the ensuing infection was confirmed to be that of recombinant CaMV H17; it carried the expected *Xho*I site at 1642 and the *Eco*RI site at 6043.

Reaction of CM1841, D4, and Cabb-B virus to Cabb-B antiserum. To determine how closely related D4, CM1814, and Cabb-B were serologically, we compared the three purified viruses with each other in an immunosorbent assay using Cabb-B antiserum. The concentrations of the purified viruses were varied from 10 to 1,000 ng/ml. D4, CM1841, and Cabb-B could all be detected at the lowest concentration, 10 ng/ml. This test indicated that the three viruses, or any recombinant between them, could be detected with equal sensitivity in an immunosorbent test using Cabb-B antiserum.

Host response to viral strains: parental types. The severity of CaMV D4 infection in *D. stramonium* is influenced by the environment. Optimal conditions for disease development occurs in the greenhouse from November to March. In the summer months, symptoms are milder and the virus concentration is lower. To compare one test with another, it was essential to standardize conditions. For this reason, tests with *D. stramonium* as a host were done in a growth chamber set for an 8-h day at 18°C. Although infection of *N. bigelovii* appears to be less affected by environment than that of *D. stramonium*, most of the tests with it were done in the greenhouse from the end of November to the middle of January.

Figure 3 illustrates the host response to infection with D4, CM1841, or Cabb-B. Local lesions on *D. stramonium* and *N. bigelovii* appeared 12 to 15 days after inoculation. On *D. stramonium*, D4 induced large, chlorotic lesions (Fig. 3A), whereas CM1841 and Cabb-B induced smaller, necrotic lesions (Fig. 3B). On *N. bigelovii*, D4 and Cabb-B induced local lesions that were chlorotic, whereas CM1841 induced no visible response.

Systemic symptoms of D4 infection developed on both hosts approximately 3 to 4 weeks after inoculation. D4 severely distorted *D. stramonium* leaves (Fig. 3D) and caused a mild mottle in *N. bigelovii*. CM1841 did not induce systemic symptoms in either host. Cabb-B induced a few widely spaced necrotic spots in systemically infected *D. stramonium* leaves 4 to 5 weeks postinoculation (Fig. 3C), but no symptoms developed in systemically infected *N. bigelovii*.

Although Cabb-B induced systemic symptoms in *D. stramonium*, the concentration of the virus was very low. No virus could be detected by immunosorbent tests when leaf disks were punched randomly from systemically infected leaves. The virus could be detected only if individual necrotic spots were collected, pooled, and then tested. Virus could be back inoculated from lesions on systemically infected *D. stramonium* leaves to turnips to give rise to typical Cabb-B symptoms. Virus particles recovered from back-inoculated plants contained a DNA genome bearing the

TABLE 2. Disease symptoms and virus concentration in *N. bigelovii* after inoculation with recombinant strains of CaMV

CaMV strain	Local lesions	Systemic symptoms	A_{450}
D4	10[a]	10[b]	0.156[c]
Cabb-B	6	0	0.029
CM1841	0	0	0.023
H9	5	0	0.001
H16	9	0	0.005
H7	2	7	0.297
H17	9	9	0.108
H12	9	9	0.298
H13	4	0	0.004
H14	8	8	0.179
None	0	0	0.028

[a] Number of plants of 10 inoculated that gave the indicated reaction 2 to 3 weeks after inoculation.
[b] Number of plants of 10 inoculated that reacted with systemic symptoms 4 to 5 weeks after inoculation.
[c] Virus concentration was determined from tissue samples taken 6 to 7 weeks after inoculation.

restriction endonuclease map characteristic of the Cabb-B isolate.

Host response to recombinant viruses. The reaction of *D. stramonium* to the recombinant viruses and the three CaMV strains is presented in Table 1. Plants showed one of two local lesion responses. The DNA sequence that correlated with lesion type was bounded by the *Sac*I site at 5822 and the *Pvu*II site at 6318. When this DNA segment, which comprised the first half of ORF 6, was derived from CM1841 or Cabb-B, the local lesion was necrotic. If this DNA segment was derived from D4, the local lesion was chlorotic.

The DNA sequence that correlated with the ability of the virus to systemically infect *D. stramonium* also mapped to the first half of ORF 6. When a virus induced a necrotic lesion, systemic spread was restricted or could not be detected. Viruses in this category were CM1841, Cabb-B, H9, H10, H13, and H16. Occasionally such viruses as Cabb-B, H9, H13, and H16 induced chlorotic or necrotic spots on a few of the older leaves. Although these four viruses caused some systemic symptoms, the concentration of the virus in test plants was very low. When noninoculated leaves were sampled randomly, the amount of virus detected by immunosorbent tests was not different from that in healthy controls.

In contrast to the results above, when a virus induced a chlorotic local lesion on *D. stramonium*, systemic symptoms subsequently developed, and the infection could be readily detected by immunosorbent tests. Viruses in this category were CaMV D4, H7, H8, H12, H14, and H17. Each of these viruses was purified from *D. stramonium*, and its identity was confirmed by restriction endonuclease mapping.

The results obtained with *N. bigelovii* were similar to those from *D. stramonium* (Table 2). The main difference between the two hosts was that viruses that could not systemically infect *N. bigelovii* induced chlorotic, rather than necrotic, local lesions. Nonetheless, systemic spread was determined by the source of the first part of ORF 6, which is identical to results with *D. stramonium*.

Genes other than ORF 6 seemed to affect the concentration of the virus in the plant. This is illustrated by the comparison of immunosorbent tests with CaMV H7 and H17 infections in *D. stramonium* and *N. bigelovii*. Genes 1 to 5 of CaMV H7 are derived from CM1841; genes 1 to 5 of CaMV H17 are derived from Cabb-B; gene 6 is derived from D4 in both cases. The concentration of CaMV H7 should thus approximate that of CaMV H17 if titers in solanaceous hosts are determined solely by the source of ORF 6. However, CaMV H7 was consistently found at higher titers than D4, whereas CaMV H17 was consistently lower.

DISCUSSION

Previous work with pseudorecombinants of RNA viruses has shown that the host range of plant viruses, as indicated by host response, segregates as a single determinant and can be mapped to a given component in viruses with multipartite RNA genomes (23, 25). In this paper we constructed recombinant viruses between three CaMV strains to map host specificity and found that a 496-bp DNA segment within ORF 6 was associated with local lesion type and ability of CaMV to infect two solanaceous plants, *D. stramonium* and *N. bigelovii*, systemically. If this DNA segment was derived from D4, the virus induced chlorotic local lesions and systemically infected the two solanaceous hosts. Conversely, when this DNA segment was derived from CM1841 or Cabb-B, the virus induced necrotic local lesions and systemic spread was greatly impaired or could not be detected.

The necrotic local lesions of Cabb-B and CM1841 are characteristic of the hypersensitive response of resistant plants. In this type of resistance, incompatible pathogens are generally limited to the region of the lesions and the plant is protected from systemic infection. The occasional appearance of lesions on newly expanded leaves of *D. stramonium* inoculated with incompatible viruses was therefore unexpected. Some virus particles appear to have escaped from localization at the initial site of host response. Secondary infection centers again induced a host defensive response. Though this was not reported in previous observations of CaMV interaction with *D. stramonium* (20), the lower-temperature, short-day conditions we used to optimize D4 infection may have altered host resistance. This partial breakdown of hypersensitive resistance has been noted for other viruses. The common strain of tobacco mosaic virus has been reported to occasionally induce necrosis on noninoculated leaves of hypersensitive *N. tabacum* 'Sansum NN', *N. tabacum* 'Xanthi', and *N. glutinosa* a few days after the local necrotic lesions appeared (10). These hosts normally are able to suppress systemic spread of tobacco mosaic virus. A low-temperature-dependent spread to induce necrosis has also been described for red clover necrotic mosaic virus (23).

Although we have clearly shown that the first half of ORF 6 controls systemic development of CaMV in two solanaceous hosts, the mechanism of this host-pathogen interaction is unknown. Two hypotheses may explain why CM1841 and Cabb-B are unable to infect solanaceous hosts. The first is that the gene VI product of CM1841 and Cabb-B is dysfunctional in solanaceous hosts. Tobacco mosaic virus, for example, moves systemically in barley only if brome mosaic virus is present, an indication that brome mosaic virus complements some defective function of tobacco mosaic virus for cell-to-cell movement (15). If the region VI protein was participating in cell-to-cell movement of virus, as we have hypothesized previously (8), the protein might not function as effectively in tobacco or *Datura* spp. as it does in *Brassica* spp.

A second hypothesis is that CM1841 and Cabb-B are prevented from moving systemically in solanaceous hosts by an active defensive response in the host. The classic example of this type of host-pathogen interaction again concerns tobacco mosaic virus, which is unable to systemically infect tobacco varieties which carry the N gene for a hypersensitive response (17).

The latter hypothesis, that an active host response blocks the spread of CM1841 and Cabb-B, is supported by two lines of evidence. Lung and Pirone (20) observed that CM1841 could proceed through the complete infection cycle in inoculated *D. stramonium* leaves, including the elaboration of typical viral inclusions and limited cell-to-cell spread. No function of CM1841, with the possible exception of cell-to-cell movement, is defective in *D. stramonium*, yet the virus is never detected in uninoculated leaves. However, in our work there also is evidence that D4 was originally sieved out of a mixture of CaMV strains by passage through *D. stramonium* (25a). This indicates that D4 is unable to complement other CaMV strains in *D. stramonium*. Complementation might be expected if incompatible CaMV isolates were merely defective in a tentative function for cell-to-cell movement. These observations indicate that incompatible CaMV isolates such as CM1841 and Cabb-B induce a host defensive response in *Nicotiana* spp. and *D. stramonium* and that ORF

6 in some manner determines the outcome of this host-pathogen interaction. In the case of a compatible host-virus interaction, the gene VI protein may suppress the hypersensitive reaction of the host.

A comparison of the putative amino acid sequence (derived from the DNA sequence) of the first half of ORF 6 of CaMV D4 with that of CM1841 has revealed an unusual number of amino acid exchanges (unpublished data). Furthermore, it has been observed that an unusual amount of variation occurs in this region for other strains of CaMV that have been sequenced. Such a high degree of variation in such a localized region of the viral genome suggests that it plays some crucial role in the establishment of successful infections and as a consequence may be under stringent selection pressure.

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